

What is claimed is:

1. A method for detecting a metabolic disorder in an individual, comprising:

(a) contacting a sample comprising:

(i) one or more metabolically indicative enzymes and

(ii) one or more metabolic analytes,

with one or more substrates for said one or more enzymes to produce a reaction admixture, under conditions in which at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product;

(b) contacting said reaction admixture with a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said one or more metabolic analytes and said at least one product are soluble in said reagent; to produce a test sample and

(c) determining the presence or amount of said one or more metabolic analytes and said at least one product contained in said test sample using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.

2. The method of claim 1, wherein said sample is a body fluid sample.

3. The method of claim 2, wherein said body fluid sample is blood.

4. The method of claim 1, wherein said sample is dried.

5. The method of claim 1, wherein said individual is a human suspected of having a metabolic disorder.

6. The method of claim 1, wherein said individual is a neonate.

7. The method of claim 1, wherein said individual is a newborn.

8. The method of claim 1, wherein said individual is a child.

9. The method of claim 1, wherein said individual is an adult.

5

10. The method of claim 1, wherein said metabolic disorder is an inborn error of metabolism.

10

11. The method of claim 1, wherein said metabolic disorder is an acquired metabolic disorder.

15

12. The method of claim 1, wherein said metabolically indicative enzyme is selected from the group consisting of oxidoreductase, hydrolase, lyase, transferase, ligase and isomerase.

13. The method of claim 12, wherein the enzyme is a hydrolase.

14. The method of claim 13, wherein the enzyme is a biotinidase.

20

15. The method of claim 14, wherein said substrate is biocytin.

16. The method of claim 1, wherein said metabolic analyte is one or more amino acids.

25

17. The method of claim 1, wherein said metabolic analyte is an acylcarnitine or plurality of acylcarnitines.

18. The method of claim 1, wherein step (a) further comprises contacting said sample with one or more reference substrates.

30

19. The method of claim 1, wherein step (b) further comprises contacting said sample with one or more reference products.

20. The method of claim 1, wherein step (d) further comprises, prior to determining, adding one or more reference products corresponding to the at least one product .

5

21. The method of claim 1, wherein step (d) further comprises, prior to determining, adding one or more reference analytes corresponding to the one or more metabolic analytes contained in said sample.

10

22. The method of claim 1, wherein said reaction admixture is aqueous.

23. The method of claim 22, wherein said reagent is non-aqueous.

15

24. The method of claim 23, wherein said reagent comprises an organic solvent.

25. The method of claim 23, wherein said reagent comprises an alcohol.

26. The method of claim 25, wherein said reagent is methanol.

20

27. The method of claim 1, wherein said mass spectrometry is tandem mass spectrometry.

28. A method for detecting a metabolic disorder in an individual, comprising:

25

(a) contacting a vessel containing one or more substrates in dried form with a sample from said individual, the sample containing

(i) one or more metabolically indicative enzymes and

(ii) one or more metabolic analytes,

in aqueous solution;

30

under conditions wherein at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product;

(b) adding a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said one or more metabolic analytes and at least one product are soluble in said reagent;

(c) determining the presence or amount of said one or more metabolic
5 analytes and said at least one product in the resultant mixture using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.

10 .

29. The method of claim 28, wherein said sample is a body fluid sample.

30. The method of claim 29, wherein said body fluid sample is blood.

15 31. The method of claim 30, wherein said sample is dried.

32. The method of claim 28, wherein said individual is a human suspected of having a metabolic disorder.

20 33. The method of claim 28, wherein said individual is a neonate.

34. The method of claim 28, wherein said individual is a newborn.

35. The method of claim 28, wherein said individual is a child.

25

36. The method of claim 28, wherein said individual is an adult.

37. The method of claim 28, wherein said metabolic disorder is an inborn error of metabolism.

30

38. The method of claim 28, wherein said metabolic disorder is an acquired metabolic disorder.

39. The method of claim 28, wherein said metabolically indicative enzyme is selected from the group consisting of oxidoreductase, hydrolase, lyase, transferase, ligase and isomerase.

5 40. The method of claim 39, wherein the enzyme is a hydrolase.

41. The method of claim 40, wherein the enzyme is a biotinidase.

42. The method of claim 41, wherein said substrate is biocytin.

10

43. The method of claim 28, wherein said metabolic analyte is one or more amino acids.

44. The method of claim 28, wherein said metabolic analyte is an acylcarnitine
15 or plurality of acylcarnitines.

45. The method of claim 28, wherein said vessel further contains one or more reference substrates.

20 46. The method of claim 28, wherein said vessel further contains one or more reference analytes corresponding to the one or more metabolic analytes in said sample.

47. The method of claim 28, wherein said vessel further contains one or
25 more reference products.

48. The method of claim 28, wherein said reagent is non-aqueous.

49. The method of claim 48, wherein said reagent is an organic solvent.

30

50. The method of claim 28, wherein said reagent contains alcohol.

51. The method of claim 50, wherein said alcohol is methanol.

52. The method of claim 28, wherein said mass spectrometry is tandem mass spectrometry.

5 53. The method of claim 28, wherein said vessel is a well of a multi-well plate.

54. A method for detecting a metabolic disorder in an individual, comprising:

(a) contacting a sample comprising:

(i) one or more metabolically indicative enzymes and

10 (ii) one or more metabolic analytes,

with one or more substrates for said one or more enzymes to produce a reaction admixture, under conditions in which at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product;

(b) contacting said reaction admixture with a reagent that inhibits the ability
15 of said one or more enzymes to act on a corresponding substrate, wherein said one or more metabolic analytes and said at least one product are soluble in said reagent, to produce a test sample;

(c) contacting said reaction admixture with one or more reference products and one or more reference analytes, and

20 (d) determining the presence or amount of said one or more metabolic analytes and said at least one product contained in said test sample, with respect to said one or more reference products and one or more reference analytes, using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic
25 analytes and said at least one product correlates with presence or absence of said metabolic disorder.

55. A method for detecting a metabolic disorder in an individual, comprising:

(a) separating a sample into two sample portions, the sample comprising:

30 (i) one or more metabolically indicative enzymes and

(ii) one or more metabolic analytes;

(b) contacting each sample portion with one or more substrates for said one or more enzymes to produce two reaction admixtures, under conditions in which at

least one of said enzymes in each sample portion is capable of acting on a corresponding substrate to generate at least one product;

(c) contacting each reaction admixture with a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said reagent can be the same or different for each reaction admixture, and wherein said one or more metabolic analytes and said at least one product are soluble in said reagent or reagents; to produce a test sample and

(d) combining the two reaction admixtures to produce a test sample, and

(e) determining the presence or amount of said one or more metabolic analytes and said at least one product contained in said test sample using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.

56. A method for detecting a metabolic disorder in an individual, comprising:

(a) separating a sample into two sample portions, the sample comprising:

(i) one or more metabolically indicative enzymes and

(ii) one or more metabolic analytes;

(b) contacting each sample portion with individual vessels, each vessel containing one or more substrates for said one or more enzymes in dried form,

(c) contacting each vessel with a solution to produce two reaction admixtures, under conditions in which at least one of said enzymes in each sample portion is capable of acting on a corresponding substrate to generate at least one product;

(d) contacting each reaction admixture with a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said reagent can be the same or different for each reaction admixture, and wherein said one or more metabolic analytes and said at least one product are soluble in said reagent or reagents; to produce a test sample and

(e) combining the two reaction admixtures to produce a test sample, and

(f) determining the presence or amount of said one or more metabolic analytes and said at least one product contained in said test sample using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.

57. A method for detecting a metabolic disorder in an individual, comprising:

(a) contacting a vessel containing one or more substrates in dried form with a sample from said individual, the sample containing

(i) one or more metabolically indicative enzymes and

(ii) one or more metabolic analytes,

(b) contacting said vessel with a solution to generate conditions wherein at least one of said enzymes is capable of acting on a corresponding substrate to generate at least one product;

(c) adding a reagent that inhibits the ability of said one or more enzymes to act on a corresponding substrate, wherein said one or more metabolic analytes and at least one product are soluble in said reagent;

(d) determining the presence or amount of said one or more metabolic analytes and said at least one product in the resultant mixture using mass spectrometry,

wherein a determined presence or amount of said one or more metabolic analytes and said at least one product correlates with presence or absence of said metabolic disorder.